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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*
Thromb-X nv, Leopold-I-straat 1 bus 21, 3000 Leuven

Patents ADP number *(if you know it)* 8311607002

If the applicant is a corporate body, give the country/state of its incorporation Belgium

4. Title of the invention
Embryonic stem cell

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Thromb-X nv
Care off:
Prof. Désiré José Collen
Collingham Garden 28
London SW5 OHN

Patents ADP number *(if you know it)* 8213118001

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Prof. Désiré José Collen
Tel. 020 72449405 fax 020 72449406

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EMBRYONIC STEM CELL

FIELD OF THE INVENTION

The present invention relates to embryonic stem cells with germ line transmission capability derived interspecies non human mammalian hybrids. Such interspecies hybrid embryonic stem cells can for instance be derivable from Mus musculus X Mus spretus hybrid mice.

The invention also relates to the use of these hybrid ES cells for the generation of genetically modified mammalian animals and for the identification of quantitative trait loci (QTL) associated with species specific phenotypes, mores specifically the invention relates to the use of these hybrid ES cells for the generation of genetically modified Mus spretus mice and for the identification of quantitative trait loci (QTL) associated with species specific (Mus musculus versus Mus spretus) phenotypes.

BACKGROUND OF THE INVENTION

ES cell lines are cell lines derived from the inner cell mass (ICM) of blastocyst-stage embryos, which can be cultured and maintained in vitro under specific conditions for many passages, i.e. replating of cells onto new cell culture dishes at regular time intervals, without loss of their pluripotency. They maintain a normal karyotype and when reintroduced into a host blastocyst they can colonize the germline (1). Such cell lines may provide an abundance of pluripotent cells that can be transformed in vitro with DNA (see below), and selected for recombination (homologous or non-

homologous) of exogenous DNA into chromosomal DNA, allowing stable incorporation of the desired gene. To date, germline transmission, i.e. the transmission of the ES genome to the next generation, has however only been achieved with ES cells of certain mouse strains.

Murine embryonic stem cells were first isolated in 1981(2,3). Since then, several ES cell lines have been established and they are now widely and successfully used for the introduction of targeted mutations or other genetic alterations into the mouse genome (6, 7). Most of the germline-competent mouse ES cell lines that are in current use have been obtained in the 129 strain, and the remainder in a few other inbred strains (C57BL/6 and crosses with C57BL/6). Furthermore, ES cell lines are at best obtained in 30% of explanted blastocysts, even in the 129 strain, and success rates of around 10% appear to be closer to the norm (4, 5).

The most commonly used approach to generate chimeric animals is to inject about 10-15 isolated ES cells into the blastocoel of a host blastocyst and to allow the cells to mix with the cells of the inner cell mass. The resultant chimeric blastocysts are then transferred to recipients for rearing. Alternatively diploid aggregation using very early (8-16 cell) stage embryos and tetraploid aggregation, can be used as hosts for ES cells. Briefly, ES cells are 'sandwiched' between early stage embryos devoid of their zona pellucida, cultured overnight and implanted into a foster mother. This technique can be performed under conditions yielding either chimeric or totally ES cell-derived offspring.

Presumptive pluripotential ES cells have been isolated from a number of other species than mice, including hamster, pig, sheep, cattle, mink, rat, primate, human, chicken, marmoset, medakafish and man. In only a few instances (pig, chicken, medakafish), have these cell lines given rise to chimeras when reintroduced into blastocysts, but thus far none have given rise to germline transmission.

ES cells are maintained in an undifferentiated state by the presence of feeder layers producing various factor(s) that prevent the cells from differentiating. It has been shown that several cytokines are responsible for this effect: DIA/LIF (differentiation inhibitory activity/leukaemia inhibiting factor), interleukin-6 in combination with soluble interleukin-6 receptor, interleukin-11, oncostatin M, ciliary neurotrophic factor and cardiotrophin. It is now possible to establish and maintain ES cells in culture in the absence of feeder cells but in the presence of such factors, at least for several passages. In species other than the mouse, ES cell technology is still under development and there are no published data reporting germ line transmission in any species other than the house mouse (Mus musculus).

We show here for the first time the derivation of ES cells from hybrid blastocysts, obtained by the mating of two different species, namely Mus musculus and Mus spretus.

ILLUSTRATIVE EMBODIMENTS OF THE INVENTION.

Advances in recombinant DNA technology over the last decade have greatly

facilitated the isolation and manipulation of genes, to the point where any conceivable novel construct can be engineered, such as by fusing the promoter of one gene to the coding sequence of another, or by site-directed mutagenesis. Likewise, advances in embryo manipulation have facilitated the transfer of these novel exogenous genes into endogenous chromosomal DNA, generating transgenic animals. Transgenic animals can be generated either by injection of DNA into the pronucleus of zygotes, by introduction of (genetically manipulated) pluripotent embryonic stem (ES) cells into host "embryos", and more recently by nuclear transfer with stably transfected somatic donor cells into enucleated oocytes.

Mus spretus

Presently all mouse embryonic stem cell are derived from the Mus musculus group. ES cells have not been derived from other mouse species yet.

All Mus species have the same basic karyotype of 40 acrocentric chromosomes.The three closest known relatives of *Mus musculus* are aboriginal species with restricted ranges within and near Europe. All three species — *M. spretus*, *M. spicilegus*, and *M. macedonicus* — are sympatric with *M. musculus* but interspecific hybrids are not produced in nature. There is a complete barrier to gene flow between the house mice and each of these aboriginal species. The ability of two animal populations to live sympatrically — with overlapping ranges — in the absence of gene flow is the clearest indication that the two populations represent different species (Nevertheless, in the forced, confined environment of a laboratory cage, Bonhomme and colleagues were able to demonstrate the production of

interspecific F1 hybrids between each of these aboriginal species and *M. musculus* (31-32).

Mus spretus is a western Mediterranean short-tailed mouse with a range across the most southwestern portion of France, through most of Spain and Portugal, and across the North African coast above the Sahara in Morocco, Algeria, and Tunisia. *M. spretus* is sympatric with the *Mus. musculus. domesticus* group across its entire range. In 1978, Bonhomme and his colleagues reported the landmark finding that *M. spretus* males and laboratory strain females could be bred to produce viable offspring of both sexes. Although all male hybrids are sterile (33), the female hybrid is fully fertile and can be backcrossed to either *M. musculus* or *M. spretus* males to obtain fully viable second generation offspring.

The species Mus spretus and Mus musculus are at an evolutionary distance of 3 million years and display great genetic polymorphisms and different stress-induced phenotypes. Therefore the embryonic stem cells of the present invention are extremely usefull in the following research fields.

Mus spretus is presently used to support research in many areas including:

* Reproductive Biology Research: Fertility Defects (male progeny from outcrosses to inbred are sterile)
* Research Tools: Genetics Research (Evolution and Systematics Research)
* Research Tools: Genetics Research (Gene Mapping: Polymorphisms)
* Research Tools: Genetic Research (Cancer and TNF resistance)

Reproductive Biology Research: Fertility Defects

In the mouse, male infertility has been found in hybrids between two species, *Mus spretus* and *Mus musculus*, from which most of the laboratory strains have been established. Understanding hybrid sterility might give an insight into not only mechanism by which a new species is evolved, but also genetic regulation of gametogenesis in males (33-34).

Genetics Research (Gene Mapping: Polymorphisms)

Until a few years ago, gene mapping in the mouse was done mainly using crosses of inbred laboratory strains or RI strains. These methods were limited, however, by the low degree of polymorphism observed among laboratory strains and RI progenitors and the associated difficulty in finding a polymorphism needed for mapping. In the mid-1980s this problem was overcome through the development of interspecific crosses, which involve crosses between a laboratory strain and a distantly related species of *Mus* (35-36). The high degree of genetic polymorphism present between the parents of such a cross makes it possible to map virtually any gene in a single cross. The most commonly used parents for interspecific backcrosses (IB) are C57BL/6J (the prototypic inbred strain) and *Mus spretus*, the most distantly related mouse species that will still form fertile hybrids with laboratory mice. The interspecific cross, involving a laboratory strain (*Mus musculus*) and a distantly related species *Mus spretus*, allowed literally thousands of genes to be mapped within the same cross.

In a series of papers, Bonhomme and colleagues have already demonstrated the power of the interspecific cross for performing multi-locus linkage analysis with molecular and biochemical markers (38-39). With the large evolutionary distance that separates the two parental species, it is possible to readily find alternative DNA and biochemical alleles at nearly every locus in the genome. This finding stands in stark contrast to the high level of non-polymorphism observed at the majority of loci examined within the classical inbred lines. The significance of the interspecific cross for mouse genetics cannot be understated: it was the single most important factor in the development of a whole genome linkage map based on molecular markers during the last half of the 1980s.

Many of the major diseases affecting humans, including diabetes, cancer, epilepsy, and obesity, are not caused by single-gene mutations, but rather by the cumulative effect of mutations at several different loci. Furthermore, some of these diseases reflect a predisposition that is genetically inherited but is under significant influence from acquired somatically mutations or environmental influences. The genes that cause or predispose to such complex diseases, called quantitative trait loci (QTLs), can be dominant or recessive and act additively or epistatically to induce disease. Each QTL by itself may have only a weak effect and it is only when several QTLs are inherited by a single individual that disease or disease predisposition ensues. In general, QTL analysis requires screening of hundreds, if not thousands, of individuals and scoring them for markers scattered across the entire genome. Obviously the mouse has advantages over humans as many inbred strains are available for analysis and thousands of progeny can easily be produced by programmed breeding. (39). In a typical QTL analysis of a complex

trait, a mapping population is generated by crossing two highly differentiated progenitor strains or lines of mice. Most often, several hundred F2 or backcross progeny are tested and genotyped genome-wide.

A number of QTLs already have been identified in the mouse that produce phenotypes similar to human diseases, such as airway hyperresponsiveness (40), alcohol and morphine preference (41-42), atherosclerosis (43), epilepsy (44), blood pressure (45), obesity (46-47), high density lipoprotein cholesterol levels (48).

Chromosomal deletions have already been shown to be powerful tools in the genetic analysis of complex genomes, enabling the systematic identification and location of functional units along defined chromosomal regions. In mice, deletion complexes created by whole animal irradiation experiments have enabled a systematic characterization of functional units along defined chromosomal regions. However, classical mutagenesis in mice is impractical for generating deletion sets on a genome-wide scale.

You et. al. (49-50) have already shown that F1 hybrid ES cells of the BALB/cTa x 129/SvJae genotype and of the C57BL/6J x 129/SvJae genotype retain germline colonizing ability after exposure to levels of irradiation that induce chromosomal deletions. Not only are these deletions useful for the identification of genetic functions, but they also serve as mapping reagents for existing mutations or traits.

The very high polymorphism between Mus musculus and Mus spretus make ES cell of the Mus musculus X Mus spretus genotype ideal cell lines for the generation of radiation-induced chromosomal deletions. Between different strains of Mus musculus no more than 40% of the microsatellites are polymorphic in length. Between C57BL/6 and Mus spretus up to 90% of microsatellite length can be polymorphic in length. The availability of Mus musculus X Mus spretus hybrid ES cell lines can therefore definitely contribute to a faster and more efficient high-throughput analysis of gene function and identification of quantitative trait loci.

Cancer resistance

Different inbred mouse strains vary greatly in their susceptibility to tumour development in a variety of tissues. Intraspecific and interspecific crosses can be used to map loci that control this disposition. Crosses of Mus musculus with Mus spretus are highly resistant to tumour development in the skin, lung and lymphoid system. (51).

Mouse models using interspecific crosses between Mus spretus, which is relatively cancer resistant, and inbred strains of Mus musculus, which are relatively cancer susceptible have been used to map cancer modifier genes

Mice of C57BL6/J inbred strain develop thymic lymphomas at very high frequency after acute gamma-irradiation, while mice of several inbred strains derived from the wild progenitor of the Mus spretus species and their F1 hybrids with C57BL/6J appear extremely resistant (52).

Interspecific crosses between Mus musculus and Mus spretus can be used for the detection of strong genetic interactions between tumor modifier genes (53)

Tumor necrotic factor resistance

The SPRET/Ei mouse strain, derived from Mus spretus, exhibits an extremely dominant resistance to TNF-induced lethal inflammation (54).

Thusfar, derivation of ES cells with germline transmission capability from species other than Mus musculus has not been realized yet. We report here for the very first time the derivation of ES cells with germline transmission capability from Mus musculus domesticus X Mus spretus (C57BL/6J X SPRET/Ei (Spain)) mice.

EXAMPLE 1: Conditioned ES cell culture medium

Conditioned medium was used for derivation and culture and culture of ES cells of Mus musculus X Mus spretus hybrid mice.

The basic medium was composed of: 500 ml DMEM high glucose, 70 ml fetal bovine serum, 13ml penicillin/streptomycin, 13ml glutamine, 6.3µl β-mercaptoethanol, and 13ml non-essential amino acids. Conditioning the basic medium with Rab9 or Rab9#19 fibroblast cells.

A) ES cell culture medium preconditioned with Rab9

The basic medium conditioned by the Rab9 fibroblast cells is obtained as illustrated below. The production of this conditioned medium has already

been described in detail in patent application **(Ash Bird T2267 PCT)**. Basic ES cell medium, conditioned by confluent monolayer cultures of the Rab9 fibroblast cells (ATCC CRL-1414), is collected for 4 consecutive days and the conditioned media are pooled for use in ES cell culture. Each day 15 cm Petri dishes are refreshed with 25 ml of basic ES medium. After 4 days each 15 cm Petri dish is split at a ratio of 1 to 4. The first day after splitting, the medium is discarded. To 1 liter of conditioned basic ES medium (from the mixture of the 4 collection days), 80ml fetal bovine serum, 17ml non-essential amino acids, 20ml glutamine, 6.3µl β-mercaptoethanol, 1.25 ml insulin and 80ml basal medium is added and the pH is adjusted to 7.4. This conditioned medium contains unmeasurable level (less than 20 pg/ml) of Rab-LIF as determined with the ELISA for human LIF of R&D Systems (Minneapolis, MN, USA).

To this composition purified recombinant Leukemia Inhibitory Factor (LIF) can optionally be added, preferably rabbit LIF (Rab-LIF) disclosed in the invention (WO0200847), or alternatively commercially available LIF. Antibiotics, such as penicillin/streptomycin, and insulin, may also be included in the composition.

B) ES cell culture medium preconditioned with Rab9#19

Basic medium, conditioned by the Rab9#19 fibroblast cells, is collected for 4 consecutive days as described for Rab9 described above. To 1 liter of conditioned basic ES medium (from the mixture of the 4 collection days), 80ml fetal bovine serum, 17ml non-essential amino acids, 20ml glutamine, 6.3µl β-mercaptoethanol, 1.25 ml insulin and 80ml basal medium is added and the pH is adjusted to 7.4. Rab9#19 are Rab9 fibroblast cells which have

been stably transfected with the rabbit Leukemia Inhibitory Factor gene and which secrete up to 30 ng/ml/day of Rab-LIF in the medium as determined with the ELISA for human LIF of R&D Systems (Minneapolis, MN, USA). The production of this conditioned medium is described in detail in patent application (WO0200847)

The *rabbit fibroblast cell line* expressing rabbit LIF (Rab9#19 clone) was deposited with accession number LMBP 5479CB) on April 07, 2000 by Thromb-X (Leopoldstraat 21, 3000 Leuven, Belgium) in the Belgian Coordinated Collections of Microorganisms (BCCM) Laboratorium voor Moleculaire Biologie- Plasmidencollectie (LMBP) Universiteit Gent, K.L.Ledeganckstraat 35, 9000 Gent, Belgium.

Examples

EXAMPLE 2: Derivation and culture of Mus musculus X Mus spretus hybrid embryonic stem (ES) cells

1. Mouse strains and ES cells

ES cells were derived from the mating of the following commercially available mouse strains: C57BL6/J (The Jackson Laboratory, Bar Harbor, Maine, USA) and SPRET/Ei (Spain) The Jackson Laboratory, Bar Harbor, Maine, USA).

2. Derivation of murine ES cells

Blastocysts were obtained from the natural matings of C57BL/6J female mice with Spretus:Ei (Spain) male mice. The blastocysts were cultured with basic medium conditioned on the Rab9 #19 fibroblast cell line.

ES cells are derived from 3.5-4.5 days old blastocyst stage Mus musculus X Mus spretus hybrid embryos, which can be collected and plated individually on a 96 well dish covered with a mitotically arrested mouse embryonic fibroblast feeder monolayer. The blastocysts are allowed to attach to the monolayer, and refed every day with conditioned ES cell medium (Cfr. Bird T2267 PCT) or with ES cell medium conditioned with the Rab9#19 cell line which secreted endogenous Rab-LIF (patent application WO0200847).

After 5-6 days in culture, the inner cell mass (ICM) outgrowth is selectively removed from the (remaining) trophectoderm and replated after trypsinization with trypsin-EDTA on a 96 well dish with mitomycin arrested murine fibroblasts. Subsequently the ES cells are gradually plated on larger culture dishes. ES cells can remain undifferentiated for more than 20 passages by using conditioned ES cell medium of the present invention.

The undifferentiated character of the established ES cell lines is determined by immunochemical staining for the presence of alkaline phosphatase (Vector Laboratories Inc., Burlingame, CA), or for the absence of vimentin and cytokeratin (both Dako A/S, Denmark). Only ES cell lines which consist for more then 90% of undifferentiated cells are maintained in culture.

Fibroblast feeder layers can be obtained from murine embryos of 12.5 days post-coitus pregnant mice. The mice are sacrificed, and the uteri collected and placed in a petri dish containing phosphate buffered saline (PBS). The embryos are dissected out of the uterus and all membranes removed. The embryos are transferred into a new dish containing PBS, the head and all internal organs removed and the carcasses washed in PBS to remove blood.

The carcasses are then minced using 2 insulin syringes into cubes of 2 to 3 mm in diameter, and incubated in Trypsin-EDTA/MEM solution (10/90 V/V) at 4°C for 2 hrs. The suspension is then incubated at 37°C for 15 min, a single cell suspension made using a 5 ml pipette, and plated at 5×10^6 cells per 180 mm petri dish in 25 ml Feeder Medium.

Feeder Medium consisted of 500 ml Dulbecco's Minimal Essential Medium (DMEM), 10% fetal calf serum (FCS), 13 ml penicillin/streptomycin, 13 ml glutamine, 13 ml non-essential amino acids, 2.3 µl β-mercaptoethanol. The medium is changed after 24 hr to remove debris. After 2 to 3 days of culture the fibroblasts reaches a confluent monolayer. The plates are then trypsinized, replated on 2 petri dishes, and, when confluent, the cells of each plate are frozen in 2 vials, kept at –80°C overnight and transferred to liquid nitrogen the next day.

Table I: Efficiency of ES cell derivation from Mus musculus domesticus X Mus spretus (C57BL/6J X SPRET/Ei (Spain)) hybrid mice.

Mouse strain	Number of blastcysts explanted	Number of ES cell lines established	
		number	%
(C57BL/6J X SPRET/Ei (Spain))	27	16	59

The basic medium conditioned by the Rab9#19 fibroblast cells allows the derivation of embryonic stem cells from the C57BL/6J X SPRET/Ei (Spain) hybrid strain. After two months of culture 16 established ES cell lines are counted. This implies an overall derivation efficiency of respectively 59%.

3. Gender of the established ES cell lines

The gender of the established ES cell lines was determined by PCR and confirmed by Southern blotting with a Y-specific probe.

ES cell line No.	gender
SPR/BL6#2	female
SPR/BL6#3	male
SPR/BL6#4	male
SPR/BL6#7	male
SPR/BL6#8	female
SPR/BL6#9	male
SPR/BL6#10	male
SPR/BL6#11	female
SPR/BL6#12	male
SPR/BL6#13	female
SPR/BL6#15	female
SPR/BL6#18	female
SPR/BL6#19	female
SPR/BL6#21	male
SPR/BL6#24	male
SPR/BL6#26	male

4. Culture of ES cells

ES cells are grown to subconfluency on mouse embryonic fibroblasts mitotically arrested with mitomycin. Culture dishes are kept at 39°C in a

humidified atmosphere of 5% CO_2 in air. The ES cells are passaged every 2-3 days onto freshly prepared feeder dishes. The ES cells are fed every day with the conditioned ES cell medium.

4. Blastocyst injection of ES cell clones

The ability of the ES cells to colonize the germ line of a host embryo was tested by injection of these ES cells into host blastocysts and implanting these chimeric preimplantation embryos into pseudopregnant foster recipients according to standard procedures. The resulting chimeric offspring were test bred for germ line transmission of the ES cell genome.

ES cells of hybrid mice with an agouti coat colour were injected into host blastocysts of albino Swiss Webster or C57BL/6N mice. This allows easy identification of ES cell contribution. All ES lines tested resulted in chimaeric offspring after blastocyst injection

5. Germ line transmission after blastocyst injection

Because of the known sterility of F1 male hybrids, two female (SPR/BL6#3 SPR/BL6#18) and only 1 male (SPR/BL6#2) ES cell lines were injected into the recipient blastocysts.

Although both female as well as the male ES cell line were able to generate chimeric offspring after blastocyst injection, only the female ES cell lines showed the capability to pass the ES cell genome to the next generation (Table 1). Germline transmission from the Mus spretus as well as from the C57BL/6J genome was observed.

Table II: Production of chimeric mice after injection of Swiss Webster or C57BL/6N blastocysts with Mus musculus domesticus X Mus spretus (C57BL/6J X SPRET/Ei (Spain)) ES cells, which were derived and cultured with basic medium conditioned on Rab9#19 fibroblasts.

ES cell line No.	Passage No.	# blasts injected	# pups born	#chimeras	germline
SPR/BL6#3	6		27	20	M1 & F1
SPR/BL6#2	7		16	6	
SPR/BL6#2	9		2	2	
SPR/BL6#3	8		5	2	
SPRBL6#18	6		3	8	F1

M: germline transmission via male chimeras

F: germline transmission via female chimeras

Alternatively medium conditioned by the Rab9 fibroblast cells could be used to derive embryonic stem cells with germline transmission capability from Mus musculus domesticus X Mus spretus (C57BL/6J X SPRET/Ei (Spain)) hybrid mice.

Mus musculus X Mus spretus (C57BL/6J X SPRET/Ei (Spain)) ES hybrid ES cells can be used to induce gene alteration by homologous or non-homologous recombination in the Mus spretus genoom.

Mus musculus X Mus spretus (C57BL/6J X SPRET/Ei (Spain)) ES hybrid ES cells can also be used for the expression or overexpression of genes in a Mus spretus background.

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EMBRYONIC STEM CELL

CLAIMS

1) Embryonic stem cell (ES), characterised in that it is a rodent interspecies hybrids cell.

2) The ES cell of claim 1, that it is a murid interspecies hybrid cell.

3) The ES cell of claim 1, or 2, characterised in that said cell is derived of the inner cell mass of blastocyst-stage hybrid embryo.

4) The ES cell of claims 2 or 3, from interspecies hybrids of the genus mus.

5) The ES cell of any of the claims 1 to 4, wherein one of the species is Mus musculus.

6) The ES cell of claim 5, wherein the other species is selected from the group consisting of Mus macedonicus, Mus spicilegus, and Mus spretus.

7) The ES cell of claim 5, from the interspecies hybrids Mus musculus X Mus spretus.

8) The ES cell as in any of the claims 4 to 7, wherein Mus musculus is a strain selected of the group consisting of Mus musculus domesticus, Mus musculus castaneus, Mus musculus L, Mus musculus castaneus, Mus musculus domesticus Rutty, Mus musculus molossinus and Mus musculus musculus.

9) The ES cell as in any of the claims 4 or 8, wherein the other species is a species of the group consisting of Mus caroli, Mus laboratorius, Mus cooki, Mus cervicolor, Mus booduga and Mus dunni.

10) The ES cell of any of the claims 1 to 9, wherein said interspecies hybrid stem cells have germ line transmission capability.

11) The ES cell as in the claims 1, 3, 5 or 10, from an hybrid wherein one of the species is Mesocricetus auratus.

12) The ES cell as in the claims 1, 3, 5 or 10, from the hybrid Mesocricetus auratus X Mus musculus.

13) The ES cell as in the claims 1, 3, 5 or 10, from the hybrid Rattus rattus X Mus musculus.

14) The ES cell as in the claims 1, 3, 5 or 10, from Mus musculus X Mus spretus hybrid mice.

15) The ES cell from claim 14, with germline transmission capability.

16) The ES cell as in the claims 1, 3, 5 or 10, from C57BL/6J X SPRET/Ei (Spain) hybrid mice.

17) The ES cell claim 16, with germline transmission capability.

18) A cell line established by any of the ES cells from claim 1 – 17.

19) The use of cell line of claim 17, for use in the generation of chimeric or ES cell derived animals.

20) The use of cell line of claim 17, for gene alteration by homologous or non-homologous recombination.

21) The use of cell line of claim 17, for the generation of animals with gene alteration via germ line transmission.

22) The use of cell line of claim 17, for the study or isolation of (novel) genes.

23) The use of cell line of claim 17, for the expression or overexpression of genes.

24) The use of cell line of claim 17, for the identification of quantitative trait loci.

EMBRYONIC STEM CELL

ABSTRACT

Present invention demonstrates the derivation of ES cells from hybrid blastocysts, obtained by the mating of two different non human mammalian species. The invention also relates to the use of these hybrid ES cells for the generation of genetically modified mammalian animals and for the identification of quantitative trait loci (QTL) associated with species specific phenotypes.

PCT Application
PCT/BE2004/000015